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HEALTH IMPLICATIONS OF 2,3,7,8-TETRACHLORODIBENZODIOXIN (TCDD) CONTAMINATION OF RESIDENTIAL SOIL

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Running Title: TCDD Levels in Soil

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NOTICE

The enclosed document constitutes the final version of the deliberations by the Centers for Disease Control (CDC) on the risk of various concentrations of 2,3,7,8-tetrachlorodibenzodioxin in soil. The document will be published in the scientific literature. It has been submitted to the Journal of Toxicology and Environmental Health. Copies of consultants' comments can be obtained by writing to:

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It will not be possible to honor telephone requests. We would appreciate it if you would therefore send all requests or other concerns you might have to us in writing.

Vernon N. Houk, M.D.
Director
Center for Environmental Health

Evaluating the Human Health Risk of Varying Concentrations of 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) Levels in Soil[1], [2]

Summary

From the available literature dealing with the toxic effects of 2,3,7,8-tetrachlorodibenzodioxin (TCDD), only reports on a few chronic feeding studies in rodents can be used for risk assessment calculations. The smallest lower confidence bound on the virtually safe dose by the linear derived multistage model using an added cancer risk of 1/1,000,000 is calculated to be 28 fg/kg b.w./day. This calculation is based on data for hepatocellular carcinoma or neoplastic nodules (female rat, Kociba et al., 1978; Squire review (EPA personal communication)). The increased cancer risk of 1/1,000,000 based on

[1] The following abbreviations have been used in this document:

- b.w. = body weight
- wk = week
- fg = femtograms
- ppb = parts per billion
- ppt = parts per trillion
- pg = picograms
- ppm = parts per million

[2] Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

data for tissue less sensitive than liver would not be expected to occur until doses as high as 1428 fg/kg b.w./day were administered. The corresponding levels for an increased risk of 1/100,000 are 276 fg to 14.3 pg/kg b.w./day (Figure 1 and 2). These calculations assume that a linear dose-response relationship exists for carcinogens (such as TCDD) that based on current evidence are thought to be primarily promoters. The dose response curve for promoters may, however, not be linear, causing an overestimate of the risk. The model was used on a hypothetical basis, and the cancer risk for TCDD should be reevaluated as the data base enlarges. Human exposure would primarily occur by the dermal and the oral route.

To estimate human TCDD intake after exposure to TCDD-contaminated soil in residential areas, we calculated estimates for dermal, ingestion, and inhalation doses. With these estimates (the assumptions on which they are based are outlined in the text), the best estimate of a daily dose at 1 ppb in residential soil (assuming uniform distribution of TCDD in soil at 1 ppb) is calculated to be 44.6 pg/day (or 636.5 fg/kg b.w./day for a person weighing 70 kg). In consideration of the range of the estimated VSD and because of the unlikelihood that all of the conservative exposure assessment assumptions will be realized on a continuous or lifetime basis, we have concluded that residential soil levels greater than 1 ppb TCDD pose a level of concern. The appropriate degree of concern for which management decisions are made should also consider an evaluation of the specific circumstances at each contaminated site.

Exposure in contaminated residential areas would be greater than in only occasionally frequented commercial areas. In residential areas, levels at or

above 1 ppb of TCDD in soil cannot be considered safe and represent a level of concern. (Houk V. Testimony before the Subcommittee on Oversight and Investigations of the Committee on Energy and Commerce of the House of Representatives, November 19, 1982.) In certain commercial areas, higher levels may present an acceptable risk to non-occupationally exposed individuals. On ranges and pastures, however, lower soil levels may still be of concern, since TCDD accumulates in the tissues of grazing cattle and rooting swine.

Background

In the early 1970's, a waste oil dealer in Missouri disposed of waste material containing 2,3,7,8-tetrachlorodibenzodioxin (TCDD) in high concentration by mixing this material with salvage oil and spraying it on dirt roads and riding arenas. The contamination of the riding arenas by TCDD was established in 1975 (Carter et al., 1975). Until recently, investigators had not realized the extent to which several other areas, many of them residential, were also contaminated with TCDD. Thus far, concentrations measured in soil in these areas have ranged from less than 1 ppb to over 1,000 ppb. Once it was determined that TCDD was present in residential areas, it had to be decided what level represented an unacceptable risk to the population living in these contaminated areas. This document presents a detailed review of the initial determination made by the Centers for Disease

Control (CDC) and includes suggestions and comments on the report made by a group of consultants (Appendix I). Where appropriate, consultants' comments have been directly incorporated in the document.

The method used for conducting a risk assessment and identifying a level of concern for TCDD in soil is complicated by many uncertainties--namely, insufficient data on the toxicology of TCDD, insufficient information about exposure of people to soil, and insufficient information about intake of TCDD by humans from such soil.

Since ambient concentrations of TCDD in water or air will usually not make a significant additional contribution to TCDD intake for populations living in significantly contaminated areas, we have not addressed these contributions in this document.

Introduction

Much has been written about TCDD, and it is not the purpose of this document to review all available literature. For an overview, the reader is referred to recent reviews (Kimbrough 1980; National Research Council of Canada, 1981). Primarily, toxicology information useful for risk assessment will be reviewed here. In making risk assessments, investigators must consider the possible routes of human exposure and the average daily dose or the total lifetime dose. Although some adverse health effects have been

observed in humans after exposure to TCDD, the dose these individuals received was not quantified. The only available dose-response data were obtained in animal studies. A critical feature of TCDD is that some animal species are much more susceptible to the toxic effects of TCDD than others. Since no dose-response information is available from human exposures, it is not known which animal species most nearly approximates human responses, although it appears that humans may not be the most sensitive species. From experience with other substances the guinea pig is often more sensitive than most other species.

Some scientists have even claimed that, with all of the exposure humans have received in occupational situations, humans must not be very susceptible to the toxic effects of TCDD, and the main health effect in humans is chloracne (May, 1973). Such far reaching statements seem inappropriate, however, since no information is available on how much TCDD the exposed workers who developed chloracne actually absorbed systemically and since chloracne can be either (1) part of a systemic disease or (2) produced locally by applying TCDD to the skin, with no toxic levels being absorbed into the body (Bauer et al., 1961). It is also too early to conclude what the long-term effects of chronic low-level exposure in humans will be. Furthermore, in estimating the risk that TCDD poses for the general population, we must consider children, females of childbearing age, the aged, and the infirm.

When given as a single dose, TCDD has a half-life of 12-30 days in small laboratory animals. After repeated dosing, it accumulates in the body and is stored in adipose tissue and to some extent, in the liver and other organs. The study by Kociba et al. (1978) indicates that at some dosage levels the rat has higher levels in the liver than in adipose tissue. In vivo and in vitro studies with radiolabelled TCDD demonstrate that the substance is not readily metabolized by cells. In animal studies, however, unidentified metabolites of TCDD have been found in urine and bile, with the high pressure liquid chromatography (HPLC) elution time of the metabolite varying in different species (Neal et al., 1982).

TCDD produces a multitude of toxicological effects. These effects are reviewed in the articles listed under "Selected References." TCDD is extremely toxic (oral LD_{50} less than 5 mg/kg b.w.) in most species tested. The effect of TCDD is delayed--animals given a single dose may not die until 40 days later. With repeated dosing, TCDD is toxic at much lower daily dosage levels, and the toxic effects of TCDD appear to be cumulative. From a risk assessment standpoint, fetotoxicity and reproductive dysfunction caused at very low dosage levels in rodents and subhuman primates and carcinogenicity in rodents are critical. Long-term feeding studies are available for these two toxicological end points in rodents. On the other hand, no long-term studies have been made to determine what the no-observed-effect level for immunotoxicity is in the monkey, the rat, or the guinea pig, nor have cancer studies been conducted in species other than rodents.

In a 2-year feeding study in rats (Kociba et al., 1978), the no-observable-effect level was 0.001 ug/kg/day. In one study the oral LD_{50} in rats was reported as 44 ug/kg. The ratio between the oral LD_{50} and a long-term daily no-effect level in rats is 44,000. If this same ratio were applied to guinea pigs, and if the oral LD_{50} in guinea pigs of 0.6 ug/kg is used, a no-effect level for chronic studies in guinea pigs might be calculated to be 0.016 ng/kg/day. A similar calculation can be made by using data obtained with monkeys (the lowest effect level is used here, since a chronic no-effect level has not been determined).

$$\frac{LD_{50} \text{ (McConnell, Moore, Dalgard, 1978)}}{\text{Effect level (Allen et al., 1979)}} \qquad \frac{\text{50-70 ug/kg}}{1.8 \times 10^{-3} \text{ ug/kg/day}} = 27{,}700\text{-}38{,}888$$

TCDD is highly lipophilic, degrades rapidly on exposure to ultraviolet light if hydrogen donors are available, does not readily migrate through soil, and appears to be only slightly taken up by root plants; furthermore, only a few strains of soil bacteria can degrade it at a very slow rate. The half-life of TCDD in soil is not known. The earlier reports stating that its half-life is less than a year appear to be erroneous.

The concentrations at which TCDD still causes toxic effects are difficult to imagine. For instance, 1 ug=10^{-6}g, 1 ng=10^{-9}g, 1 pg=10^{-12}g, and 1 fg=10^{-15}g. There are 28 g in one ounce. One molecule of TCDD weighs

$$\frac{322 \text{ g/mole}}{6.023 \times 10^{23} \text{ molecules/mole}} = 5.35 \times 10^{-22} \text{ g/molecule}$$

This translates into 1.8×10^{6} molecules per fg.

Sources and Occurrence of TCDD

In the production of 2,4,5-trichlorophenol from tetrachlorobenzene, TCDD is formed as a contaminant. Subsequent cleanup of 2,4,5-trichlorophenol results in industrial waste (still bottom residue) that contains high (up to 1,000 ppm) concentrations of TCDD. The products made from 2,4,5-trichlorophenol, such as 2,4,5-T (trichlorophenoxyacetic acid) and hexachlorophene, and 2,4,5-trichlorophenol itself, may still be contaminated with trace amounts of TCDD.

TCDD and other chlorinated dibenzodioxins may also be formed during combustion. These chemicals have been identified in soot, fly ash, and many other products that were burned (Bumb et al., 1980; Buser et al., 1978). The concentrations found have usually been in parts per trillion (nanogram per kilogram) (Eiceman et al., 1980; Kooke et al., 1981).

Because of the inherent toxicity of TCDD, levels in the environment at concentrations in the parts per trillion-parts per billion range may be of toxicological significance. Methods have only recently been developed to measure such low concentrations. Therefore, no systematic monitoring for TCDD in the environment has been conducted (Norstrom et al., 1982).

Soil levels measured recently in contaminated sites in Missouri have ranged from less than 1 ug/kg (ppb) to over 1 mg/kg (ppm) (U. S. Environmental Protection Agency (EPA), unpublished information). Concentrations measured in

contaminated riding arenas in 1971 and 1974 were 30 mg/kg and after excavation around 1 mg/kg, respectively (Carter et al., 1975). EPA is now characterizing soil TCDD levels at sites where 2,4,5-trichlorophenol was produced or used to make 2,4,5-T and other chemicals (Anonymous, 1983).

Metabolism in Animals

The toxicokinetics are reviewed by Neal et al. (1982).

a. Absorption

In Sprague-Dawley rats given a single oral dose of 1.0 ug [^{14}C] 2,3,7,8-TCDD/kg b.w., absorption from the intestinal tract was estimated to be around 83% (Rose et al., 1976). With repeated oral dosing at 1.0 ug/kg/day (5 days/wk x 7 wk), absorption was similar to that observed for the single oral dose (Rose et al., 1976). With a much larger single oral dose, 50 ug/kg b.w., about 70% of the dose was absorbed by rats (Piper et al., 1973). In studies where TCDD was administered to rats by gavage in acetone:corn oil (1:25 or 1:9), absorption from the gastrointestinal tract ranged from 70-85% (Rose et al. 1976; Piper et al., 1973). When TCDD was administered to rats in the diet at 7 or 20 ppb (0.5 or 1.4 ug/kg/day) for 42 days, 50 to 60% of the consumed dose was absorbed (Fries and Marrow, 1975).

Poiger and Schlatter (1980), using hepatic concentrations in rats 24 hours after dosing as an indicator of the amount absorbed, found a linear relationship between nanograms of TCDD administered in 50% ethanol (for doses of 12 to 280 ng, equivalent to 0.06 to 1.4 ug/kg) and the percentage of the dose in hepatic tissues (36.7 to 51.5%). Only about half of the amount of TCDD given in ethanol was absorbed when TCDD was administered in an aqueous suspension of soil.

Information on the absorption of 2,3,7,8-TCDD through the skin is found only in a study by Poiger and Schlatter (1980), and no information on absorption through the respiratory tract was found. Poiger and Schlatter (1980) administered 26 ng TCDD in 50 ug methanol to the skin of 6 rats. After 24 hours, the liver contained 14.8 $\pm$ 2.6% of the dose. Application of TCDD in a soil/water paste decreased hepatic TCDD to about 2% of the administered dose. It is not clear whether absorption from the skin would be as rapid as that from the stomach. These studies suggest that small amounts of TCDD are absorbed through the skin, but they give no information about the rate of absorption and distribution of TCDD in the body over time.

b. Distribution

Piper et al. (1973) used a single oral dose of [^{14}C] TCDD to study distribution and excretion of TCDD in male Sprague-Dawley rats. Most of the radioactivity (53.2%) was excreted via the feces, but the urine and expired air accounted for 13.2 and 3.2%, respectively. Analysis of the tissues after

3 days showed liver and adipose tissue to contain the highest percentage of the dose per gram of tissue, with 3.18 and 2.60%, respectively.

Rose et al. (1976) also examined the distribution of [^{14}C] TCDD in rats. Twenty-two days after a single oral dose of 1.0 ug/kg, liver and adipose tissue had each retained about 1.2% of the dose. With repeated oral doses of 0.01, 0.1, or 1.0 ug [^{14}C]-TCDD/kg/day, Monday through Friday for 7 wk, the liver and fat contained most of the body burden of TCDD, accounting for 50 and 10 times more [^{14}C] activity, respectively, than the rest of the carcass. As a result of these studies, they concluded that the rate of TCDD accumulation in the body, after single and repeated exposure, was largely accounted for by the rate of accumulation in liver and fat. With a single oral dose, no radioactivity was detected in either the urine or expired air, indicating that most, if not all, of the elimination of TCDD and/or its metabolites was via the feces. With repeated oral doses, the [^{14}C] activity was also excreted primarily through the feces, but significant amounts were found in the urine. Male rats given 1.0 ug/kg/day of TCDD for 7 wk excreted an average of 3.1% of the cumulative dose in the urine, whereas the female rats excreted an average of 12.5% in the urine (Rose et al., 1976).

Studies performed by Van Miller et al. (1977) on rhesus monkeys and rats with single intraperitoneal doses of tritiated TCDD showed that although rats had over 40% of the TCDD in the liver 7 days after dosing, the monkeys had only about 10% in the same organ at that time, and a greater percentage was

found in skin and muscle tissue. In two strains of mice, the liver contained about 35% of an administered dose of TCDD 1 day after oral or intraperitoneal administration (Manara et al., 1982).

Kociba et al. (1978) found that female rats maintained on a daily dietary TCDD intake of 0.1 ug/kg/day for 2 years had an average TCDD content of 8100 ppt (ng/kg) in fat and 24,000 ppt (ng/kg) in the liver. Rats given 0.01 ug/kg/day had an average of 1700 ppt (ng/kg) of TCDD in the fat and 5100 ppt (ng/kg) in the liver. For both of these daily dosages, the liver-to-body-fat ratio of TCDD was 3:1. At the lowest dose level of 0.001 ug/kg/day, both fat and liver contained an average of 540 ppt 2,3,7,8-TCDD. Kociba et al. (1978) presented evidence that a steady-state in rats had been reached after 13 weeks of TCDD feeding.

McNulty (1982) reported that 2 years after administering a single oral dose of 1 ug/kg of TCDD to an adult rhesus macaque monkey, tissue levels of the compound were 100 ppt (ng/kg) in adipose tissue and 15 ppt (ng/kg) in liver. These results indicate that prolonged retention of TCDD may occur in this species. The retention in humans is not known.

TCDD has also been shown to pass the placenta of rats and mice (Moore et al., 1976; Nau and Bass 1981; Applegren et al., 1983), and it is excreted in milk (Moore et al., 1976).

TCDD is a potent inducer of hepatic microsomal mixed function oxidase enzymes with the lowest effective single dose in the rat of 0.002 ug/kg b.w. (Kitchin and Woods, 1979).

c. Excretion

The following discussion assumes that elimination is a first-order process, except in the guinea pig, in which elimination may follow zero-order kinetics (Gasiewicz and Neal, 1979).

TCDD is slowly excreted from the bodies of all small laboratory species tested, with a half-life in the body for single doses of 10 to 43 days. In the golden Syrian hamster, the mammalian species least sensitive to the acute toxicity of TCDD, excretion occurs readily through both the urine (41% of total excreted radioactivity) and feces (59% of total excreted radioactivity) (Olson et al., 1980). In all other species tested so far, excretion occurs mainly through the feces, with only minor amounts of TCDD metabolites found in the urine (Rose et al., 1976; Gasiewicz and Neal, 1979).

Metabolites of TCDD have been detected in the bile and urine of golden Syrian hamsters after single oral or intraperitoneal doses (Olson et al., 1980) and in the bile of dogs after repeated direct introduction of the chemical into the duodenal lumen (Poiger et al., 1982).

Poiger and Schlatter (1980) and Ramsey et al. (1979) demonstrated biliary excretion of several metabolites of [^{14}C] TCDD by rats after repeated oral dosing. The metabolites were tentatively identified as glucuronides of hydroxylated TCDD. The amounts of metabolites found were small.

Mutagenesis and Cell Transformation

The results of *in vitro* and *in vivo* mutagenesis studies are summarized by Kociba and Schwetz (1982), Hay (1982), and Rogers et al. (1982). In tests with salmonella strains, TCDD has been reported to be mutagenic in the salmonella typhimurium strain TA 1532 and the escherichia coli strain Sd-4. In other laboratories strains TA 1532, TA 1535, TA 1537, and TA 1538 have not yielded positive results. On the other hand, prophage induction in escherichia coli K-39 was positive, but a dominant lethal study in rats was not. Cytogenetic studies in rat bone marrow were negative or questionable.

Hay (1982) recently was able to show cell transformation in kidney hamster cells. Although in a number of cell lines it was possible to induce arylhydrocarbon hydroxylase, there have been almost no observations of cell toxicity. Only in a mouse teratoma cell were Knudson and Poland (1982) able to induce keratinization by the addition of TCDD. A similar transformation occurs in sebaceous glands when chloracne develops and the cells of the sebaceous glands are transformed into squamous cells.

In many instances there appears to be an association between such metaplasia and the development of cancer. However, metaplasia, that is, cell transformation, may also occur without necessarily progressing into cancer such as the transformation of columnar into squamous epithelium. Cell transformation per se does therefore not represent conclusive evidence that TCDD is an initiator of carcinogenesis. For instance, in vitamin A deficiency, squamous metaplasia develops in the trachea, bronchus, and the pelvis of the kidneys, the uterus and the pancreatic ducts (Pinkerton 1977). Although vitamin A has been claimed to protect against the development of certain carcinomas, its lack can certainly not be considered to be an initiator of cancer.

Mechanism of Action

It is not clear why some species are more susceptible to the toxic effects of TCDD and why the target organs vary in different animal species. The toxicity of this chemical apparently depends on the fact that the lateral positions of the molecule are occupied by chlorine or bromine in the case of brominated compounds (Poland and Glover, 1973).

Induction of hepatic aryl hydrocarbon hydroxylase (AHH), cytochrome P-448, and a number of other enzymes appears to be controlled by a single gene in the mouse known as the Ah locus (Poland and Glover, 1975). In inbred strains of mice, AHH inducibility is inherited as a simple autosomal dominant trait. Poland et al. (1976) observed a small pool of displaceable high

affinity binding sites in the hepatic cytosol of rats and mice which has the in vitro binding properties predicted for a receptor for induction of AHH. Polycyclic hydrocarbons such as 3-methylcholanthrene compete with TCDD for the binding site, but compounds which do not induce this enzyme (thyroxine, steroids, phenobarbital, DDT) do not compete. As would be expected if this binding site represents the receptor, the displaceable binding of [^{3}H]TCDD to the hepatic cytosol of genetically responsive mice is much greater than that of nonresponsive mice. Recent work indicates that the receptor binds TCDD, and the complex translocates to the nucleus (Greenlee and Poland, 1979). Using isoelectric focusing, Gustaffson and co-workers (Carlstedt-Duke et al., 1978) have recently demonstrated the presence of a protein in hepatic cytosol with a molecular weight of 136,000 after partial digestion with trypsin, which has a high specific binding affinity for TCDD (10^{-9}) similar to that reported by Poland et al. (1976). This protein could be detected in AHH-responsive mice, but not in nonresponsive mice.

All of these data are consistent with the hypothesis that chlorinated dibenzodioxins and polycyclic aromatic hydrocarbons combine with a cytosolic receptor, which enters the nucleus and produces coordinate induction of a number of enzymes. In the rat, mouse and a number of other species, these enzymes include AHH and other cytochrome P-448 associated monooxygenase activities, glucuronyl transferase, DT diaphorase, ornithine decarboxylase, and aldehyde dehydrogenase. The nature of the proteins controlled by the

genome would depend on the tissue and on the species. Recent studies of *in vivo* covalent binding in rats (Poland and Glover, 1979) demonstrate that covalent binding to DNA (6 pmol TCDD per molecule of nucleotide residue) is 4 to 6 orders of magnitude lower than that of most chemical carcinogens and the binding of DNA is equivalent to one molecule of TCDD per DNA of about 35 cells. TCDD induced oncogenicity is, therefore, most likely not caused through a mechanism of covalent binding to DNA and somatic mutation.

It has thus far not been conclusively demonstrated that all toxic effects, specifically lipid peroxidation, are mediated through the cytosol receptor. A number of related aromatic compounds, such as certain isomers of the halogenated naphthalenes, biphenyls, and dibenzofurans, have similar biological activities if the lateral positions are occupied by chlorine or bromine (Goldstein, 1980).

Animal Toxicology

Teratogenesis and Reproduction

TCDD is a teratogen in several strains of mice at doses much lower than most other teratogens. Cleft palate and kidney anomalies predominate. When CF-1 mice (between 14 and 41 mice/dose group) were dosed by gavage on day 6 through 15 of gestation with five doses of TCDD ranging from 0.001 to 3 ug

TCDD/kg b.w./day, no significant effects were observed at any dosage of TCDD on implantation sites per litter, live fetuses per litter, sex ratio, fetal body weight, fetal crown-rump length, or skeletal abnormalities. The percent of resorptions increased significantly ($p < 0.05$) at doses of 1 ug TCDD/kg b.w./day. Cleft palate increased significantly at 1 and 3 ug TCDD/kg b.w./day, and the incidence of bilaterally dilated renal pelvis was significantly increased at 3 ug TCDD/kg b.w./day (Smith et al., 1976). The authors concluded that the rate of malformations in CF-1 mice was not significantly increased at doses less than or equal to 0.1 ug TCDD/kg b.w./day.

In rats, TCDD produces fetotoxic effects at lower doses than are required for teratogenic effects and increases the incidence of intestinal hemorrhage and edema, kidney anomalies, and intestinal hemorrhage (Murray et al., 1979).

Murray et al. (1979) studied the effects of 0, 0.001, 0.01 and 0.1 ug TCDD/kg b.w./day given in the diet of Sprague-Dawley rats over three generations. No significant toxic effects were observed in the F_0 generation during 90 days' treatment before mating. Both fertility and neonatal survival were significantly reduced in the F_0 generation, and neonatal survival was reduced in the F_1 generation at doses of 0.1 ug TCDD/kg b.w./day. At 0.01 ug TCDD/kg b.w./day, fertility was significantly reduced in the F_1 and F_2 generations but not in the F_0 generation. In addition, daily doses of 0.01 ug TCDD/kg b.w./day reduced litter size, decreased fetal and neonatal survival, and decreased growth. Doses of 0.001 ug TCDD/kg b.w./day had no effect on fertility, litter size, postnatal body

weight, or neonatal survival. Therefore, Murray et al. (1979) concluded that doses of 0.1 and 0.01 but not 0.001 ug TCDD/kg b.w./day produced deleterious effects on reproduction through three generations of rats. However, Nesbit and Paxton (1982) in their analysis of these data concluded that the lowest dose still affected reproduction. A review of these data in conjunction with this report leads to the conclusion that there was insufficient evidence for an effect at 0.01 ug/kg b.w./day (Appendix II).

Khera and Ruddick (1973) observed dose-related decreases in average litter size and pup weight of Wistar rats treated with doses greater than 0.25 ug TCDD/kg b.w./day. Survival was significantly decreased at 0.5 ug TCDD/kg b.w./day, and no pups survived at doses of 1.0 ug TCDD/kg b.w./day. The Sprague-Dawley rat appears to be more susceptible to the toxic reproductive effects of TCDD than the Wistar rat.

Allen et al. (1979) have reported adverse effects of TCDD on reproduction in nonhuman primates (rhesus monkeys). A decrease in serum estradiol and progesterone levels was noted after a 7-month exposure to diets containing 500 ng/kg of TCDD (approximately 18 ng/kg b.w./day). The length of the menstrual cycle and menstruation, however, were normal. After a 7-month exposure to TCDD, the females were mated with untreated males. Three of eight females conceived; however, two aborted.

In a second experiment, Allen et al. (1979) fed female rhesus monkeys a diet containing 50 ng/kg (approximately 1.8 ng/kg b.w./day). After a 6-month exposure, serum estradiol and progesterone levels were normal. Mating of eight treated females with untreated males resulted in six pregnancies, from which there were four abortions and two normal births. All eight control females conceived and had normal births. This daily dose of 1.8 ng/kg would be equivalent to a total dose of 378 ng/kg b.w.

Allen et al. (1979) and McNulty (1982) studied the effect of TCDD in rhesus monkeys. Whereas Allen et al. (1979) produced effects on reproduction in monkeys with a total dose of roughly 500 ng/kg given over a 6-month period, McNulty observed similar effects with a single dose of 1 ug/kg (Table 1) but not with a single dose of 200 ng/kg. These data suggest that total doses in the low nanogram per kilogram body weight range may not affect reproduction in monkeys. Thus far, a no-effect level on reproductive outcomes in the rhesus monkey has not been reported nor have any long-term (several years) or multigeneration studies been conducted in this species.

Immunotoxicity

Thigpen et al. (1975) treated 4-wk-old specific pathogen-free male C57BL/6Jfh mice once weekly for 4 wk with 0.5, 1, 5, 10, or 20 ug TCDD/kg b.w. (equivalent to 0.07, 0.14, 0.71, 1.43, or 2.86 ug TCDD/kg b.w./day). A significant decrease in body weight gain was observed in the 2.86 ug TCDD/kg b.w./day group. Doses of 0.14 ug TCDD/kg b.w./day or greater for 4 wk,

followed by infection with Salmonella borne, significantly increased mortality and decreased the time from infection to death. TCDD treatment did not affect the mortality of the mice caused by Herpesvirus suis infection. Thymic atrophy and liver pathology were present at 2.86 and 1.43 ug TCDD/kg b.w./day but not at lower doses. These results are difficult to interpret; furthermore, the mice may have had preexisting immunity to the herpes virus.

In contrast, Vos et al. (1978) and Thomas and Hinsdill (1979) did not observe any impairment in the response of TCDD-treated mice to challenges with Listeria monocytogenes. Male Swiss mice were given 50 ug TCDD/kg b.w./wk for 4 wk. The mice were then challenged with Listeria, and viable Listeria were measured in the spleen 2 days later. TCDD treatment did not affect spleen Listeria counts nor did it impair the ability of peritoneal macrophages to reduce nitro-blue tetrazolium to formazan. Thus, TCDD treatment did not seem to affect macrophage function in mice.

Vos et al. (1978) studied the susceptibility of TCDD-treated mice to Escherichia coli endotoxin. Three- to four-wk-old specific pathogen-free Swiss mice were dosed by gavage once a week for 4 wk with 0, 1.5, 5, 15, or 50 ug TCDD/kg b.w. (equivalent to 0, 0.21, 0.71, 2.14, or 7.14 ug TCDD/kg b.w./day). Two days after the final dose of TCDD, the mice were injected intravenously with E. coli endotoxin, and mortality was assessed after 48 hours. A TCDD dose-related increase in sensitivity to E. coli endotoxin was observed. With 10 ug of endotoxin, the no-effect dose was 0.71 ug TCDD/kg b.w./day over 4 wk.

Thomas and Hinsdill (1979) studied 5- to 6-wk-old mice from specific pathogen-free mothers fed diets containing 1, 2.5, or 5 ug of TCDD/kg^{-1}. The offspring were injected with a range of doses of Salmonella typhimurium lipopolysaccharide endotoxin, and mortality was assessed over 14 days. A TCDD dose-related increase in mortality was observed with a no-effect level of 1.0 ug TCDD/kg^{-1} diet (0.15 ug/kg b.w./day^{-1}) when 40 ug of endotoxin was administered.

Vos and Moore (1974) assessed cell-mediated immune functions in rats exposed to TCDD prenatally or prenatally and postnatally. Fisher-344 rats received 0, 1, or 5 ug TCDD/kg b.w. on days 11 and 18 of gestation. In some cases, the pups remained with treated mothers during lactation, whereas other groups suckled nontreated mothers. The transformation of spleen cells by the phytomitogen phytohemagglutinin on a per cell basis was significantly reduced in rats postnatally exposed to TCDD. The response of spleen cells to phytohemagglutinin on a per spleen basis was reduced to 72% of that of controls in rats from dams dosed with 1 ug TCDD/kg b.w. on days 11 and 18 of gestation. The response of thymus cells to phytohemagglutinin (on a per cell basis) was significantly reduced in rats from dams given 5 ug TCDD/kg b.w., and the response to concanavalin A was significantly decreased on a per thymus basis.

Graft-versus-host reactions and allograft rejection have also been studied. Vos and Moore (1974) reported prolonged allograft rejection times in rats and reduced graft-versus-host reactions in rats and mice after exposure

to TCDD. The rats were exposed to TCDD through the dam's milk. The offspring of dams that were dosed with 5 ug TCDD/kg b.w. on days 11 and 18 of gestation and on days 4, 11, and 18 of lactation showed significant effects in both the allograft rejection time and graft-versus-host reactions, whereas lower dosages did not cause any observable effects. Mice treated with 0.14, 0.71, and 3.57 ug TCDD/kg b.w./day showed a decrease in graft-versus-host reactivity.

In addition, Faith et al. (1978) and Faith and Luster (1979) reported that TCDD suppressed delayed hypersensitivity in Charles River albino rats given 5 ug TCDD/kg b.w. on day 18 of gestation, then 5 ug TCDD/kg b.w./wk. Some pups were cross-fostered onto untreated dams to provide a prenatal exposure group. Oxazolone was used as the contact sensitizing agent, and the radiometric ear assay also was used. A suppression in the delayed hypersensitivity reaction was observed in all exposure groups.

Thomas and Hinsdill (1979), using dinitrofluorobenzene as the sensitizing agent, studied the effects of TCDD exposure on delayed hypersensitivity reactions by measuring the increase in ear thickness. Swiss Webster mice were fed diets containing 0, 1, 2.5, and 5.0 ug TCDD/kg. A significant suppression in the delayed hypersensitivity reaction was observed in offspring from dams on the 5 ug/kg diet (about 0.75 ug TCDD/kg b.w./day) tested at 5 wk of age.

In summary, some studies suggest that the humoral immune function may be affected in rodents (Vos and Moore, 1974; Thomas and Hindsdill, 1979; Vecchi et al., 1980), although Faith et al. (1978) and Faith and Luster (1979) were

not able to substantiate these findings. TCDD effects on the cellular immune function have been more consistently shown, although no chronic studies have been conducted to more precisely characterize this effect.

The studies done in rats and mice suggest that the developing immune system is more susceptible to the effects of TCDD than the adult immune system. On the other hand, in the guinea pig the thymus is the primary target organ for TCDD, and weekly doses of 40 ng TCDD/kg b.w. for 8 wk depressed the delayed hypersensitivity reaction to tuberculin (Vos et al., 1973) in adult guinea pigs.

Several other studies have been conducted in guinea pigs, rats, and mice to evaluate the immune response, but they do not give any additional information about dose response. Although the immunotoxicity of TCDD is a serious health effect in animals--apparently present at low doses of TCDD exposure--we cannot use these data in risk analysis at this point, since no adequate dose-response data exist.

Carcinogenicity and Other Chronic Toxic Effects

A study by Van Miller et al. (1977) in Sprague-Dawley rats suggests that TCDD was carcinogenic. However, only 10 rats per group were used. The results of that study are summarized in Table 2. It is surprising that no tumors at all were observed among the controls. Since there was no increase

of any particular tumor but only an increase of total tumors, these data are not very useful. The findings were not statistically significant, and the number of animals tested was small.

Subsequently, Kociba et al. (1978) reported a study in which groups of 50 male and 50 female Sprague-Dawley rats (Spartan substrain) were fed diets containing 22, 203, and 2,193 ng/kg of TCDD for 2 years. This is equivalent to daily doses of 0.001, 0.01, and 0.1 ug TCDD/kg b.w. The total intake of TCDD for rats surviving 24 months would be 0.73, 7.3, and 73 ug TCDD/kg b.w. for the three treated groups. The controls consisted of 86 male and 86 female rats.

Numerous toxicologic effects were observed at 0.1 ug TCDD/kg b.w./day. These effects included increased mortality; decreased body weight gain; depressed hematological parameters; increased urine levels of porphyrins and δ-aminolevulinic acid; increased serum enzyme activity for alkaline phosphatase, γ-glutamyl transferase, and glutamic-pyruvic acid transaminase; and morphological changes in hepatic, lymphoid, respiratory, and vascular tissues.

At 0.01 ug TCDD/kg b.w./day, liver toxicity, focal pulmonary alveolar hyperplasia, and, in females, increased urinary porphyrin excretion were noted. No toxic effects of significance were reported in rats exposed to 0.001 ug TCDD/kg b.w./day for 24 months. A summary of the tumor incidence observed by Kociba et al. (1978), for tumors whose incidence in treated and control rats was significantly different, is presented in Table 3.

Toth et al. (1979) conducted studies in Swiss/H/Riop mice. Three groups of 45 male mice were given weekly doses (by gavage) of 7.0, 0.7, or 0.007 ug TCDD/kg b.w. for 1 year, then studied for their entire lifetimes. An equal number of control mice (45) were given the TCDD vehicle (sunflower oil) each week. The incidence of liver tumors was significantly increased in the 0.7 ug TCDD/kg b.w./wk group (48% tumor incidence) compared with that of the control group (18% tumor incidence). The incidence of liver tumors observed in the 7.0 ug TCDD/kg b.w./wk group was 30% greater but was not statistically significantly different from that of the control group. Increased mortality in this group may account for not finding a statistical significance.

The National Toxicology Program (NTP) (1982 a,b) also conducted carcinogenicity studies in rats and mice. TCDD was investigated in groups of 50 male and 50 female Osborne-Mendel rats and 50 male B6C3F1 mice (0.01, 0.05, or 0.5 ug/kg/wk) and in 50 female mice (0.04, 0.2, or 2.0 ug/kg/wk). TCDD was suspended in a vehicle of 9:1 corn oil: acetone and administered by gavage 2 x/wk for 104 wk. A dose-related depression in mean body weight gain was observed in male and female rats, compared with groups of 75 vehicle controls. Significant increases were observed in incidences of follicular-cell adenomas in the thyroid in male rats, neoplastic nodules of the liver in high-dose female rats, hepatocellular carcinomas in male and female mice, follicular adenomas in the thyroid in female mice, and toxic hepatitis related to TCDD administration in high-dose rats and mice of both sexes. Under the conditions of this bioassay, TCDD was carcinogenic for both Osborne-Mendel rats and B6C3F1 mice (Table 4). In addition, the

carcinogenicity of an acetone suspension of TCDD applied to the clipped backs of 30 male and 30 female Swiss-Webster mice 3 x/wk for 99 or 104 wk was investigated. Females received 0.005 ug TCDD/application, and males, 0.001 ug TCDD. Vehicle controls consisted of 45 mice of each sex treated with 0.1 ml acetone 3 x/wk. Mean body weights of dosed male and vehicle-control mice were less than those of untreated male controls throughout the study; for females, mean body weights were less than those of untreated controls for the first 80 wk. An increased incidence of pyelonephritis was observed in male mice exposed to acetone alone or in combination with TCDD. A statistically significant increase in the incidence of fibrosarcoma of the integumentary tissue was observed in female mice given TCDD and TCDD after DMBA compared with controls. Fibrosarcomas appeared significantly earlier in TCDD-dosed males than in vehicle controls, although the increased incidence of such tumors was not statistically significant.

In females, the incidence of fibrosarcoma in the integumentary system was 30% (8/27 mice) in the treated group and 5% (2/41 mice) in the controls. In the males, the incidence of the same tumor type was not significantly different--21% (6/28) among the exposed mice and 7% (3/42) among the controls.

If the cancer studies conducted by Kociba et al. (1978) and by the NTP (1982 a,b) are compared, it is evident that the tumors in the liver are produced at quite similar dosage levels. (See also "Considerations for Risk Assessment.") Although increased incidence of tumors in other organs was observed by the NTP and by Kociba et al., the target organs varied in the two studies. This may be caused, in part, by differences in dosing (gavage versus exposure to TCDD in ground chow) and differences in the strains of rats used.

At the CDC consultants' meeting and in written comments by one of the consultants and at a meeting in Cincinnati where the EPA criteria documents on TCDD in water and air were reviewed (EPA dioxin meeting July 27-29, 1983), it was pointed out that the carcinomas of the lungs and upper respiratory tract in the study by Kociba et al. (1978) could conceivably have been caused by direct contact of TCDD-contaminated feed particles with the respiratory tract. The reasons for this are as follows:

In the Kociba study, TCDD was offered to the rats in ground chow. Rats exposed to ground food often have food particles in their airways and lungs, and this is noted on microscopic examination. In the NTP (1982b) study, TCDD was given by gavage, and liver tumors, but not tumors of the respiratory tract, developed. The direct dose to the respiratory tract cannot be estimated in the rats that developed cancer of the respiratory tract. In localized areas, it most likely was higher than the total daily dose calculated on a body-weight basis.

Promoter versus Initiator

Although the available evidence shows that TCDD has a tumor-promoting capacity, there is, as yet, very little to suggest that it is also an initiator.

Poland et al. (1982) have recently presented evidence of TCDD tumor promotion in skin in HRS/j hairless mice, and Pitot et al. (1980) showed TCDD to be a potent tumor promoter in a two-stage model of carcinogenesis in rat liver. The amount of TCDD bound to DNA is four to six orders of magnitude less than that for other known carcinogens (Poland and Glover, 1979). However, it has not been established what possible role any metabolites of TCDD may play. Binding to DNA does not necessarily mean that the DNA is altered, nor is it possible to determine with absolute certainty that TCDD has no initiating properties.

Human Health Effects

Most of our information about the human health effects of TCDD has been obtained from studies of workers who were exposed to TCDD during the production or handling of 2,4,5-trichlorophenol and products made from this chemical; as noted previously, precise exposure data, necessary for dose-response calculations, are not available for these situations. In some plants, workers primarily developed chloracne but no systemic illness (May, 1982). Other authors have reported complaints of weight loss, easy fatigability, aching muscles, insomnia, irritability, loss of libido, and sensory changes. The liver may become tender and enlarged, and decreases in nerve conduction velocity have been reported. Total serum lipids may be increased, and the prothrombin times may be prolonged (IARC, 1977; Bauer et al., 1961; Bleiberg et al., 1964; Jensen and Walker, 1972; Oliver, 1975). Porphyria cutanea tarda has also been observed (Jirasek et al., 1976; Bleiberg et al., 1964; Poland et al., 1971).

Follow-up studies in exposed workers have not been very informative, partly because the number of workers included has been small. The largest of these groups consisted of 121 workers (Zack and Suskind, 1980).

May (1982) reported that 10 years after an incident, in which 79 workers developed chloracne because of exposure to TCDD, half of the affected subjects still had chloracne. No other adverse effects were reported.

In another episode, reported by Pazderova-Vejlupkova et al. (1981), the condition of many patients, with relatively severe early findings, improved over the years. Apparently, during 1965 to 1968, 80 workers who had been engaged in the production of 2,4,5-sodium trichlorophenoxyacetate and the butyl ester of trichlorophenoxyacetic acid became ill after TCDD exposure. A 10-year follow-up study was conducted of 55 exposed individuals. Most of the patients had initially developed chloracne, and 11 manifested porphyria cutanea tarda. About half of the patients had elevated lipids with abnormalities in the lipoprotein spectrum; two-fifths had abnormal glucose tolerance tests; one-third had elevated liver function tests; and the liver tissues from liver biopsy material of selected patients fluoresced under ultraviolet light, indicating elevated porphyrins. Most suffered from various psychological disorders. As of this date, two patients have died of bronchogenic lung carcinoma; one of liver cirrhosis; one of a rapidly developed, extremely unusual type of atherosclerosis precipue cerebri; and two patients have died from traffic injuries. No conclusions can be drawn from this list of fatalities, since these are conditions which normally occur in the general population and since there were only very few deaths. The conditions of most other patients have improved.

Hardell and Sandstrom (1979) and Eriksson et al. (1981) conducted two case-control studies in Sweden and reported an increased risk of soft tissue sarcomas in men who were exposed either to trichlorophenols or to phenoxy herbicides during their application. These authors also reported a third case-control study from Sweden which suggests that phenoxy acids and chlorophenols may also predispose to Hodgkin's lymphoma, but as yet there is little support for this theory from other sources (Hardell et al., 1981). The Swedish studies were recently summarized and discussed by Coggon and Acheson (1982), who concluded that "further research is urgently needed to confirm or refute these associations, to define the extent of the risk (if any) and to identify the carcinogen(s)."

The Swedish results could not be substantiated by Milham (1982). Preliminary results from a case-control study under way in New Zealand also have not indicated an excess risk of soft tissue sarcoma (Smith et al., 1976, 1983). The mortality rate for soft tissue sarcomas for United States males between 40-64 years of age ranges from about 5-20 per 1,000,000. This low incidence severely limits the power in some studies to detect such rare events. The Swedish studies, however, are supported by results from the United States (Honchar and Halperin, 1981; Cook, 1981) in workers producing these chemicals. In the United States four follow-up studies were conducted among workers exposed to 2,4,5-trichlorophenol or 2,4,5-T (Cook et al., 1980; Ott, Holder, and Olson, 1980; Zack and Gaffney, 1983; Zack and Suskind, 1980). All of these investigators concluded that there were no excess deaths due to any cause. However, each of three cohorts had one death due to soft

tissue sarcoma. Honchar and Halperin (1981) reviewed the four studies and noted that in the four merged cohorts there were a total of 105 deaths, 3 of which (2.9%) were due to soft tissue sarcoma. On the basis of national rates of death for men aged 20 to 80, only 0.07% of deaths due to soft tissue sarcoma would have been expected. Recently, another person in one of the four cohorts died because of a soft tissue sarcoma (Cook, 1981; Ott, personal communication), bringing the total to four deaths due to soft tissue sarcoma in the four merged U.S. cohorts. Microscopic review of tissue sections from these tumors and three additional cases was recently done. Two of the four cases with documented evidence of exposure and 3 additional cases that did not have documented evidence of exposure were confirmed to represent soft tissue sarcoma. (Fingerhut et al., manuscript in preparation.) The question has also been raised as to whether it is appropriate to merge these separate cohorts.

Thiess et al. (1982) reexamined a cohort of 74 persons who had been exposed to dioxin 27 years earlier during an uncontrolled reaction at a trichlorophenol production facility. Overall mortality (21 deaths) did not differ in this group from the rate expected in three external reference populations or from that observed in two internal comparison groups. Of the 21 deceased persons, 7 had cancer (ICD No. 140-199), compared with an expected 4.1 ($p=0.14$). Three deaths due to stomach cancer (ICD No. 151), at ages 64, 66, and 69 years, were found, compared with 0.6 expected ($p=0.024$) from regional mortality data. One stomach cancer occurred among 148 individuals in the two comparison cohorts.

Despite the increasing number of reports suggesting a positive association of soft tissue sarcomas and exposure to chemicals known to be contaminated with chlorinated dibenzodioxins, several of the CDC consultants expressed caution or skepticism. For example:

"Epidemiological reports have included prospective cohort studies (some with more than 3000 person-years) in which exposures have been well documented, even if not well quantified, in which no excess mortality or malignancies were observed. The few case-control reports that suggest an excess risk of cancer are compromised by their poorly documented and obviously heterogeneous exposures, uncertainty that controls were appropriately selected, and the potential for introduction of a serious error through recall bias. Additionally, one of the studies mentioned in the review by Coggon and Acheson deals with phenoxyacetic acid herbicides known not to be contaminated with TCDD.

The possibility of a relationship between TCDD exposure and various soft tissue neoplasms, mesenchymal tumors, and sarcomas has been raised. Grouping of these diverse tumor types is not appropriate. However, the question of a possible causal relationship of TCDD and soft tissue tumors needs to be explored further." [Note: Since these tumors are extremely rare the negative studies done to date may have insufficient power to detect them.]

Another consultant added:

"A number of case-control and follow-up studies have been conducted in worker populations. Each suffers from one or more deficiencies: (1) a lack of sufficient measured exposure, (2) a lack of sufficient time to develop disease between the exposure and the study, (3) a population size too small to reasonably find cases of soft tissue sarcoma, or (4) possible lack of contamination of the commercial product with dioxin."

These comments illustrate some of the present controversies. It is hoped that larger epidemiologic studies, such as the study based on a follow-up of workers in the National Institute for Occupational Safety and Health (NIOSH) dioxin registry, will resolve these issues.

Useful information from studies of health effects following environmental exposure is sparse (Pocchiani et al., 1979). After an explosion at the ICMESA plant in 1976 (Hay, 1976), children in Seveso developed chloracne (Reggiani, 1980). Results of some liver function tests were elevated in that population (Reggiani, 1980), and the incidence of abnormal results of nerve conduction tests was reported to be statistically significantly elevated in subjects with chloracne (Fillipini et al., 1981). A child in Missouri (Carter et al., 1975) who played in dirt contaminated with 30 ppm TCDD in some areas of a riding arena had a hemorrhagic cystitis. Claims have been made that exposure to

2,4,5-T contaminated with TCDD has resulted in an increased incidence of spontaneous abortions, malformations, cancer, and other health problems (Kelby et al., 1980; Consultative Council on Cogenital Abnormalities, 1978). Since the studies reporting such results have severe methodologic limitations, additional well-designed studies need to be conducted before any conclusions can be drawn about these health effects in the general population.

One problem with all of the human studies, including reports from workers, is that direct objective measurement of exposure is not available. In situations where no systemic health effects were observed, absorption of TCDD may have been minimal or nonexistent. For instance, the highest soil level in Zone A in Seveso close to the factory was 55 ppb (ug/kg), whereas levels on the vegetation ranged from nondetected to 15.8 ppm. Of the 44 vegetation samples analyzed, 33 had less than 1 ppm TCDD. Most of the vegetation was removed early, and people in the area closest to the factory were evacuated 2 wk after the event and were warned not to eat vegetables from their gardens. The area where people are living now (Zone B) has soil levels below 0.15 ppb. Although several comments were received that exposure in Seveso was substantial, soil contamination levels in Missouri are 10-1000 times higher than in Seveso. For additional information on environmental contamination in different parts of the world, the reader is referred to Reggiani (1980).